EFFECT OF HYPEROXIA ON HEMOGLOBIN, SERUM PROTEIN AND GAS METABOLISM IN ADRENALECTOMIZED RATS

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Translation of "Vliyaniye giperoksii na gemoglobin, belki syvorotki i gazoobmen u adrenalektomirovannykh krys," Patologicheskaya Fiziologiya i Eksperimental'naya Terapiya, No. 3, May-June 1974, pp. 39-43

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16. Abstract Increased oxygen pressure (1, 2 and 3 gage atm) for 15-17 days, one hour each day, caused statistically unreliable shifts in erythrocyte and hemoglobin content in intact rats, whereas a 16.3% decrease in peripheral blood erythrocyte content and a 14.8% decrease in hemoglobin were observed in adrenalectomized rats. Both increased oxygen pressure and adrenalectomy tended to decrease hemoglobin I, II, III and IV fraction content. In adrenalectomized rats, fraction III rose only during 1 and 2 gage atm. Both adrenalectomized and intact rats developed dysproteinema during adaptation to hyperoxia.

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ANNOTATION

In case of the action of increased oxygen pressure of 1, 2 and 3 gage atm for 1 hour for 15-17 days, adrenalectomized animals, in distinction from intact animals, displayed a reduction in peripheral blood erythrocytes by 16% and of hemoglobin by 14% on the average; with adrenalectomy alone, without any hyperoxybaria, these shifts constituted only 5-6%. By electrophoresis of erythrocyte hemolysates on agar, adrenalectomy was found to lead to a tendency to reduction in the content of fractions I, II and III and to elevation of fraction IV of hemoglobin. During adaptation of adrenalectomized rats to increased oxygen pressure of 1-2 gage atm, the content of fraction III increased, but with 3, it also fell. In adrenalectomized rats, dysproteinemia was observed, expressed in hyperglobulinemia, particularly after adaptation to 3 gage atm.

3 tables, 1 illustration, 17 bibliographical entries.

EFFECT OF HYPEROXIA ON HEMOGLOBIN, SERUM PROTEIN AND GAS METABOLISM IN ADRENALECTOMIZED RATS

V. P. Dudarev*

Despite the great number of works on hyperoxia, the mechanisms/39** of action still have not been completely revealed, which is explained by its ambiguous effect on an organism -- its presence is necessary for oxidation-reduction reactions to occur; however, at increased pressure, oxygen is a strong toxic agent for living organisms.

In the pathogenesis of disturbances, arising in an organism under increased oxygen pressure (IOP), the adrenals have an important role, as organs of neurohormonal regulation of function. Some authors have demonstrated that adrenalectomy increases the resistance of animals to IOP [12, 16, 17], and others have demonstrated a reduction in resistance to IOP in adrenalectomized rats [10]. In this case, the effect of IOP on the blood system and certain types of metabolism of adrenalectomized rats has not been studied, in connection with which, this work has been carried out.

Method

The tests were carried out on Wistar rats, mainly females. Adrenalectomized animals, receiving a 1% NaCl solution, were subjected to IOP (1-3 gage atm), for 1 hour a day, for a period of 15-17 days. An alkali CO_2 absorber was placed in a chamber, 20 l in volume. Hemoglobin heterogeneity was investigated by electro-

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**Numbers in the margin indicate pagination in the foreign text.

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TABLE 1

CHANGE IN AMOUNT OF HEMOGLOBIN, ERYTHROCYTES, OXYGEN CONSUMPTION AND WEIGHT OF ADRENALECTOMIZED RATS AS A RESULT OF HYPEROXIA (M±m)

Test conditions	Hemoglobin (in g%)		Erythrocytes (in 10^6)		O_2 consumption (in ml/min/kg)		Weight (in g)	
	Before	After	Before	After	Before	After	Before	After
Adrenalectomy (18)	15.6±0.6	14.7±1.1***	7.0±0.3	6.7±0.7*	28.1±2.7	21.5±3.6***	191±16	201±16
" + 100% O_2 (12)	15.1±0.7	14.2±0.6**	7.8±0.5	6.0±0.8**	32.0±3.9	29.1±1.0*	179±20	184±19
"+ 1 gage atm O_2 (12)	15.4±0.6	14.1±0.1***	7.7±0.5	6.7±0.3**	32.0±3.9	30.4±1.0*	178±10	190±13*
"+ 2 " " O_2 (12)	15.8±1.1	13.7±0.9***	6.4±0.8	6.4±0.5	29.4±4	18.1±2.1***	190±20	177±24
"+ 3 " " O_2 (12)	14.2±2.3	12.1±1.0***	7.4±0.7	6.2±0.8***	30.2±3.0	15.3±4.4[1] ($P<0,01$)	160±25	166±27

[1] O_2 consumption at 3 gage atm by 5 rats.

NOTE: Here and in Tables 2 and 3 significant difference is from initial data (Table 1) or from control (Table 2 and 3), *P<0.05; **P<0.01; ***P<0.001. The figures in parenthesis are numbers of animals.

phoresis of erythrocyte hemolysates on agar, in a phosphate buffer, pH 7.2. At the result of endosmosis, the hemoglobin moved toward the cathode. The blood serum proteins were fractionated on paper, in a veronal-acetate buffer. The content of the fractions was determined by elution. All determinations were carried out in the morning, the day before the first IOP session and 12-14 hours after it. Blood was obtained from the tip of the tail, in determination of the total number of erythrocytes and amount of hemoglobin and by means of decapitation for electrophoretic analyses. Oxygen consumption was determined according to Holden, in the modification of N. I. Kalabukhov [8].

Results and Discussion /40

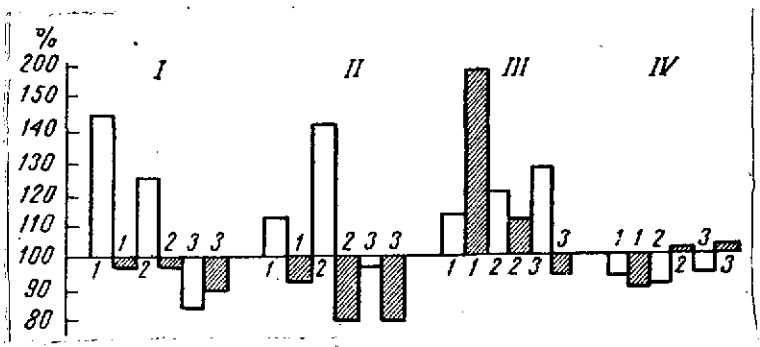
Under normal conditions, adrenalectomy leads to weakening of an entire series of physiological and biochemical processes. In our tests (Table 1), the content of hemoglobin decreased by 5.8%, erythrocytes by 4.3% and oxygen consumption by 23.5%, as a result of adrenalectomy, with statistically insignificant changes in body weight; this is evidence of negligible anemia, and corresponds to data in the literature.

Under IOP conditions, the hemoglobin content decreased, and this reduction became more pronounced with increase in hyperoxybaria, reaching 14.8% at 3 gage atm. The erythrocyte content decreased by almost the same amount (16.3%). We carried out a study of the blood 12-14 hours after IOP sessions; therefore, the possibility of deposition of erythrocytes, as a consequence of hyperoxybaria, was slight. However, there is some fractional

TABLE 2

CHANGE IN RELATIVE CONTENT OF HEMOGLOBIN FRACTIONS IN ADRENALECTOMIZED RATS AFTER HYPEROXIA (M±m)

Test Conditions	Hemoglobin Fraction (in %)			
	I	II	III	IV
Control (150)	4.9±1.1	7.9±1.4	8.1±1.4	79.1±2.9
Adrenalectomy (12)	4.5±1.0	6.0±2.3*	6.6±1.6**	83.0±3.2**
" + 100% O_2 (18)	4.5±0.4***	6.9±0.2***	15.3±2.2***	72.4±7.3**
" + 1 gage atm O_2 (12)	4.8±0.4*	7.3±0.4	16.3±1.0***	71.4±1.3**
" + 2 " " O_2 (10)	4.8±0.7	6.3±0.8***	9.0±0.8*	79.6±1.8
" + 3 " " O_2 (13)	4.4±1.3	6.3±2.0*	7.6±3.0	81.7±4.0**



Change in hemoglobin fraction content after adaptation to increased oxygen pressure (IOP): light bars, intact rats; cross-hatched, adrenalectomized; Roman numerals designate hemoglobin fraction and Arabic, IOP (in gage atm).

TABLE 3

CHANGE IN RELATIVE BLOOD SERUM PROTEIN FRACTION CONTENT OF ADRENALECTOMIZED RATS AFTER HYPEROXIA (M±m)

Test Conditions	Total Protein	Albumins	α_1-globulins
Control (intact) (41)	7.5±0.3	52.1±2.8	8.5±1.2
Adrenalectomy (14)	7.7±0.6*	48.7±3.3**	8.6±1.3
" + 100% O_2 (12)	7.3±0.3*	48.8±2.4*	11.1±0.9***
"+ 1 gage atm O_2 (12)	8.1±0.3*	52.7±1.6	9.1±1.3*
+ 2 " " O_2 (10)	6.7±0.4*	51.1±4.4	9.3±2.1*
3 " " O_2 (5)	7.8±0.5	44.5±2.9***	11.6±1.2***

Test Conditions	α_2-globulins	β-globulins	γ-globulins
Control (intact) (41)	8.0±0.8	16.0±0.9	13.6±1.0
Adrenalectomy (14)	8.4±1.0	18.6±1.9*	16.2±2.5*
" + 100% O_2 (12)	8.0±0.9*	16.0±1.3*	15.3±1.4*
" + 1 gage atm O_2 (12)	6.9±1.0*	17.2±1.1**	14.4±1.4*
" + 2 " " O_2 (10)	8.6±2.0*	15.7±2.6	15.0±2.5
" + 3 " " O_2 (5)	10.3±0.8***	18.8±1.9***	14.6±2.0

possibility of stasis of the blood in the capillaries, since the tonus of the vessels and musculature is weakened after adrenalectomy [15]. Moreover, IOP itself reduces the blood flow rate [7]. A reduction in blood formation under IOP conditions has been observed in intact animals and man, only after the acute or toxic effect of IOP [7, 9]. In the conditions which we selected for adaptation to IOP, shifts in quantity of erythrocytes and hemoglobin were statistically insignificant in intact rats. Therefore, it can be assumed that, under the conditions of our tests, the animals did not experience "hyperoxic anoxia" [11] since the activity of the circulatory system increases under hypoxia, even in adrenalectomized rats [1-3].

In addition to the quantitative changes in erythrocyte and hemoglobin content, the qualitative composition of the hemoglobin is disrupted by IOP and adrenalectomy (Table 2). The fact that the relative content of the small fractions was decreased and that of fraction III, only after addition of 100% oxygen, as well as 1 and /41 2 gage atm increase deserves attention. However, it also decreased under 3 gage atm. In comparison with intact animals [4], it is not an increase in the small fraction content, but a tendency towards a decrease in them, amounting to about 20%, for example, at 2-3 gage atm, which is characteristic of adrenalectomized rats under IOP conditions (see figure).

It follows from the data presented that adrenal hormone insufficiency affects the level of individual hemoglobin fractions. Under physiological conditions, these hormones apparently stimulate

synthesis of fractions I and II and inhibit synthesis of fraction III. Since the physiological role of the individual hemoglobin fractions of rats has not been studied, it can only be assumed that shifts in the ratios of these fractions in various actions apparently are connected with conformational changes or with disruption of synthesis.

In breathing tank oxygen without excess pressure and after IOP at 1 gage atm, the oxygen consumption of adrenalectomized rats decreased less, than in breathing atmospheric air (see Table 1). However, with increase in IOP to 2 gage atm, the oxygen consumption decreased by 38.5% and, to 3 gage atm, by 49.4%. This caused a disruption of tissue metabolism, both as a consequence of adrenalectomy and the drop in the permissive role of corticosteroids and in connection with the specific effect of IOP on oxidative enzymes, which has been demonstrated by many investigators on intact animals in cultures and in tissue sections [10].

The blood serum protein spectrum also reflects the stress on physiological systems. Under normal test conditions, adrenalectomy /42 was accompanied by a hypoalbuminemia and hyperglobulinemia (Table 3) which is in agreement with data in the literature. In both intact [5] and adrenalectomized rats, upon adaptation to IOP, dysproteinemia was observed. The total protein content in blood serum changed negligibly in this case, although it was statistically significant. For our test conditions, these changes are connected with both adrenalectomy and with the effect of IOP on the protein synthesizing function of the corresponding organs, protein catabolism and redistribution of the liquid portion of the blood, as a

consequence of disruption of membrane permeability.

It should be noted that the functional activity of the adrenals, mediated through the hypothalamo-hypophyseal and sympathetic nervous systems, becomes of especially great importance under hyperoxic conditions [6, 13, 14]. Confirming data in the literature, fewer animals among the adrenalectomized rats died (7 of 24), than among intact ones (11 of 24), under repeated IOP of 3 gage atm. Death of rats was not observed at lower IOP values. It is important that, microscopically, almost no inflammation of the lungs, in the form of pneumonia and hemorrhage, which are the most frequent causes of death of animals and appearance of the toxic effect of IOP, was observed in the former. Of course, catecholamines have a definite role in the genesis of these complications in intact animals.

In evaluating the general state of the animals and the shifts noted here, it should be emphasized that, upon adaptation to IOP, adrenalectomized rats do not react by an increase in activity of the systems, the characteristics of which we have studied.

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